GSI Equine IL-10 ELISA Kit- Plasma/Serum DataSheet

IL-10, also known as human cytokine synthesis inhibitory factor (CSIF), is an anti-inflammatory cytokine that is produced by T cells, NK cells, mast cells and macrophages (1,2,3). It is capable of inhibiting synthesis of pro-inflammatory cytokines like IFN-γ, IL-2, IL-3, TNFα and GM-CSF made by cells such as macrophages and regulatory T-cells. IL-10 also displays potent abilities to suppress the antigen presentation capacity of antigen presenting cells. IL-10 initiates signal transduction by binding to a cell surface receptor complex consisting of IL-10 RI and IL-10 RII (1). Binding of IL-10 leads to the activation of Jak1 and Tyk2, which phosphorylates Stat-3 (1,4). The anti-inflammatory activity of IL-10 is due to its ability to block signaling through other cytokine receptors, notably IFNγ receptor, by upregulating expression of SOCS-1 (1,4). In addition, IL-10 promotes T cell tolerance by inhibiting tyrosine phosphorylation of CD28 (5,6). IL-10 is an important negative regulator of the immune response, which allows for maintenance of pregnancy (1). In contrast, increased IL-10 levels contribute to persistent Leishmania major infections (7).

References

1. Pestka, S. et al. (2004) Immunol Rev 202, 8-32.
2. Akuffo, H. et al. (1999) Clin Exp Immunol 117, 529-34.
3. Grimbaldeston MA, et al (2007). Nat. Immunol. 8 (10): 1095–104.
4. O'Shea, J.J. and Murray, P.J. (2008) Immunity 28, 477-87.
5. Akdis, C.A. and Blaser, K. (2001) Immunology 103, 131-6.
6. Akdis, C.A. et al. (2000) FASEB J 14, 1666-8.
7. Von Stebut, E. (2000) Eur J Dermatol 17, 115-22.

PRINCIPLE OF THE ASSAY

This is a quick ELISA assay that reduces time to 50% compared to the conventional method, and the entire assay only takes 3 hours. This assay employs the quantitative sandwich enzyme immunoassay technique and uses biotin-streptavidin chemistry to improve the performance of the assays. An antibody specific for equine IL-10 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-10 present is bound by the immobilized antibody. After washing away any unbound substances, a detection antibody specific for equine IL-10 is added to the wells. Following wash to remove any unbound antibody reagent, a detection reagent is added. After intensive wash a substrate solution is added to the wells and color develops in proportion to the amount of IL-10 bound in the initial step. The color development is stopped and the intensity of the color is measured.

This package insert must be read in its entirety before using this product.

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MATERIALS PROVIDED

Description	Quantity	Description	Quantity	Description	Quantity
Antibody Precoated Plate	1	20 x PBS	1	Substrate Solution	1
Detection Antibody	1	10 x Wash Buffer	1	Stop Solution	1
Detection Agent	1	10 x Reagent Diluent	1	Datasheet/Manual	1
Standard	3				

Bring all reagents to room temperature before use.

1 x **96-well Plate precoated with equine IL-10 capture antibody-**Store at 4 ° C upon received.

Equine IL-10 Detection Antibody (60 µL) – The Detection Antibody should be stored at -20° C to -70° C in a manual defrost freezer for up to 6 months, if not used immediately. Spin to bring down the material prior to open the vial. The vial contains sufficient Detection Antibody for a 96-well plate. Vortex briefly and allow it to sit for a minimum of 15 min prior to use. Take the entire 60 µL of detection antibody to 10 mL Reagent Diluent if the entire 96-well plate is used. If the partial antibody is used store the rest at -20° C until use.

Equine IL-10 Standard (3 vials) – Equine IL-10 Standard has a total of 3 vials. Each vial contains 42 µL of the standard sufficient for a 96-well plate. The undiluted standard can be stored at -20° C for up to 2 months if not used immediately. Spin to bring down the material prior to open the tube. The vial contains sufficient equine IL-10 standard protein for three 96-well plates. Add 458 µL of Reagent Diluent to a Standard vial containing 42 µL of the standard to make the high standard concentration of 20,000 pg /ml. Vortex briefly and allow it to sit for a minimum of 15 min prior to use. A seven point standard curve is generated using 2-fold serial dilutions in Reagent Diluent, vortex briefly for each of dilution step. Store the rest of the standard at -20° C.

Detection Agent (50 µL) – Make 1:200 dilution in Reagent Diluent. If the entire 96-well plate is used, add all 50 µL of Detection Agent to 10 mL Reagent Diluent prior to the assay. The rest of undiluted Detection Reagent can be stored at 2 - 8° C for up to 6 months. DO NOT FREEZE.

20 x PBS, pH 7.3, 30 mL- Dilute to 1 x PBS with deionized distilled water and mix well prior to use.
10 x Wash Buffer, 40 mL- Dilute to 1 x Wash Buffer with 1 x PBS prior to use.
10 x Reagent Diluent, 3 mL – Prior to use dilute to 1 x Reagent Diluent with 1 x PBS and mix well.
Substrate Solution, 12 mL.
Stop Solution, 6 mL.

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Assay Procedure

1. Lift the plate cover and cover the wells that are not used using the strip provided. Vortex briefly the samples prior to the assay. Add 100 µL of plasma or serum or less to each well, and in each well add PBS to a total volume of 100 µL if the sample is less than 100 µL. Add 100 µLof standards per well and use duplicate wells for each standard or sample. Cover the 96-well plate and incubate 1 hour at room temperature.
2. Aspirate each well and wash with 1 x Wash Buffer, repeating the process two times for a total of three washes. Wash by filling each well with 1 x Wash Buffer (300 µL) using a multi-channel pipette, manifold dispenser or auto-washer. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or by inverting the plate and blotting it against clean paper towels.
3. Add 100 µL of the working dilution of the Detection Antibody to each well. Cover the plate and incubate 1 hour at room temperature.
4. Repeat the aspiration/wash as in step 2.
5. Add 100 µL of the working dilution of Detection Agent to each well. Cover the plate and incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.
6. Repeat the aspiration/wash as in step 2.
7. Add 100 µL of Substrate Solution to each well. Incubate for 10-20 minutes at room temperature. Avoid placing the plate in direct light.
8. Add 50 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well immediately, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

Precaution and Technical Notes

1. If IL-10 exceeds the upper limit of the detection, the sample needs to be diluted with PBS. Dilution factor must be used for calculation of the analyte concentration.
2. Detection Agent contains enzyme, DO NOT mass up with Detection Antibody.
3. The Stop Solution is an acid solution, handle with caution.
4. A standard curve should be generated for each set of samples assayed.
5. This kit should not be used beyond the expiration date on the label.
6. A thorough and consistent wash technique is essential for proper assay performance. Wash Buffer should be dispensed forcefully and removed completely from the wells by aspiration or decanting. Remove any remaining Wash Buffer by aspiration or by inverting the plate and blotting it against clean paper towels.
7. Use a fresh reagent reservoir and pipette tips for each step.
8. It is recommended that all standards and samples be assayed in duplicate.
9. Avoid microbial contamination of reagents and buffers. This may interfere with the sensitivity of the assay.

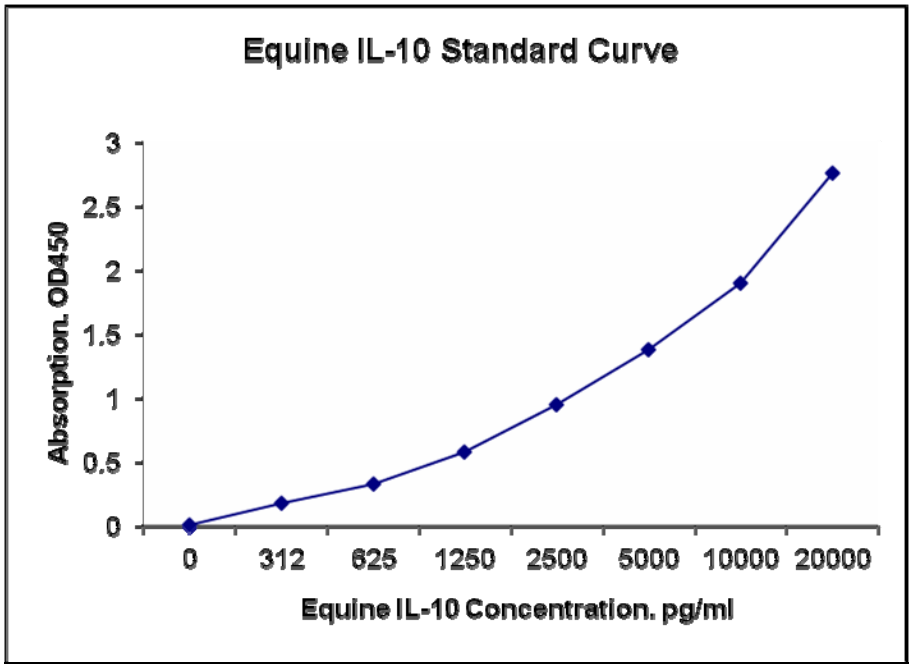
Calculation of Results

Average the duplicate readings for each standard, control, and sample and subtract the average zero (blank) standard optical density.

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the IL-10 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

The Standard Curve

The graph below represents typical data generated when using this equine IL-10 ELISA Kit. The standard curve was calculated using a computer generated 4-PL curve-fit. For this case, a Bio-Rad iMark™ Microplate Reader and a Microplate Manager 6 Software were used to generate this curve.



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Specificity

The following recombinant equine proteins prepared at 1 ng/ml were tested and exhibited no cross-reactivity or interference.
IL-1 beta, IL-2, IL-4, IL-5, IL-6, IL-8, IL-15, INFγ, TLR1, TLR2, TNF-α.

Calibration

This kit is calibrated against a highly purified yeast-expressed recombinant equine IL-10.

Detection Range

6-20000 pg/ml

Assay Sensitivity

2 pg/ml

Assay Precision

Intra-Assay %CV: 5; Inter-Assay %CV: 8

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